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Benzene, an Experimental Multipotential Carcinogen: Results of the Long-Term Bioassays Performed at the Bologna Institute of Oncology

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In 1976, a systematic and integrated project of long-term carcinogenicity bioassays began at the Bentivoglio Experimental Unit of the Bologna Institute of Oncology. The Bologna experiments proved for the first time that benzene is an experimental carcinogen. These experiments demonstrated that benzene is carcinogenic when administered by ingestion and by inhalation and that it cause tumors in the various tested animal models (Sprague-Dawley rats, Wistar rats, Swiss mice, and RF/J mice). They also showed that benzene is a multipotential carcinogen, as it produces a variety of neoplasias in one or more of the tested animal models, including Zymbal gland carcinomas, carcinomas of the oral cavity, nasal cavities, skin, forestomach, and mammary glands, as well as angiosarcomas of the liver, hemolymphoreticular neplasias, tumors of the lung, and possibly hepatomas. The Bologna experiments also indicated a clear-cut dose-response relationship in benzene carcinogenesis.

This report presents the up-to-date results of the Bologna project. The need for more experimental research aimed at assessing the carcinogenic effects of low doses of benzene, of chemical mixtures containing benzene, and of benzene substitutes is emphasized. Also recommended are more comprehensive epidemiological investigations, extended to all types of malignancies, with particular regard to lung carcinomas.

Introduction

Benzene has been produced industrially from coal since 1849 and from petroleum since 1941. At present the major source of benzene is petroleum. Benzene is one of the largely diffused and produced industrial compounds. It is a constituent of crude oil, it is present in gasoline and other fossil fuels, and it is currently produced at the rate of about 15 million tons per year (the major producers are the U.S., Japan, and Western Europe). The total global annual cycle of benzene is estimated to be 32 million tons per year (*1*).

The major use of benzene in past was in blends with gasoline. Although this use has been reduced in the United States, benzene is still extensively employed in many countries for the production of commercial gasoline. The benzene content in gasoline varies from country to country, and its range is estimated to be from 1 to 15%. Currently, benzene is used as a chemical intermediate for the production of many important industrial compounds, such as ethylbenzene (used in the production of styrene), phenol, cyclohexane, maleic anhydride, aniline, dichlorobenzenes, etc., which, in turn, supply numerous sectors of the chemical industry, especially those producing plastics, resins, elastomers, dyes, and pesticides. In the past, benzene was also used as a solvent for paints and rubber, in the production of rubber cement (widely used in the shoe and garment industries), and in the manufacture of artificial leather. It has also been used in medicine in the treatment of hemoblastomas (leukemias, polycytemia, and malignant lymphomas) and in veterinary medicine of disinfecting wounds.

Of the 32 million tons of benzene circulated globally per year, 4 million tons are estimated to be lost to the environment (*1*). The major source is motor vehicle emission and evaporation losses during handling, distribution, and storage of petrol (*2*). Burning wood and organic material also results in an appreciable release of benzene. Tobacco smoke contains benzene at levels of 47 to 64 ppm (*3*). It is believed that plant and animal matter also release benzene into the environment (*4*).

Population groups that may be exposed to benzene in-

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clude workers engaged in its production; workers in chemical industries using benzene as an intermediate; workers in industries producing materials containing benzene as a constituent (gasoline), as a solvent (rubber cement), or as an impurity (i.e., industry toluene); people living near factories producing or using benzene, or compounds containing it; tobacco smokers; and the general population (particularly in industrialized towns), as benzene is contained in gasoline, drinking water, and many other goods and is highly volatile.

Prolonged exposure to benzene causes toxic effects on bone marrow, both in animals and humans. The toxic effects on the hematopoietic system in humans have been known for about 90 years and are well documented in the literature. The classical clinical finding in benzene hematotoxicity is a decrease in the various formed elements of circulating blood (pancytopenia) as a consequence of the decrease in identifiable granulocyte, erythrocyte, and platelet precursors within the bone marrow. The association between long-term exposure to benzene and the occurrence of leukemia was suggested as early as 1928 by Delore and Borgomano (*5*), who reported a lymphoblastic leukemia in a worker who had been exposed to benzene for 5 years.

In spite of its industrial importance, widespread use, ubiquitous diffusion, the large number of people potentially exposed, and the early reports of occupational leukemias, there were no adequate epidemiological investigations nor adequate experimental research on benzene until the mid-1970s.

Knowledge of Benzene Carcinogenicity until the Mid-1970s

Human data are based, almost exclusively, on reports of a series of clinical cases of leukemia (generally in individuals with a history of benzene myelotoxicity) and on more indirect epidemiological investigation on the correlation between benzene exposure and the incidence of leukemias.

Since the first report of Delore and Borgomano (*5*), many leukemias in people exposed to benzene were the subject of case reports. Vigliani in 1976 (*6*) stated that "... an approximate estimation of the available literature, including some unpublished North Italian cases of which we have knowledge, puts the number of known cases of leukemia attributed to benzene at, at least, 150." These cases were collected and reviewed by Goldstein (*7*) (Table 1).

Acute myelogenous leukemia has been the most frequent form of leukemia associated with benzene exposure. Other forms of leukemia that have also been associated with benzene are erythroleukemia, acute monocytic leukemia, chronic myelogenous leukemia, myelofibrosis and myeloid metaplasia, thrombocytemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, and lymphomas of various types.

The majority of the cases have been found in Italy (*6,8*), France (*9–14*), and Turkey (*15,16*). Most of the cases occurred among shoemakers and garment industry workers handling rubber cement.

Table 1. Case reports of hemolymphoreticular neoplasia and correlated diseases observed in individuals exposed to benzene reported in scientific literature until the mid-1970s (*7*).

Type	No. of individuals	No. of reports
Acute myelogenous leukemia	58	28
Erythroleukemia	16	10
Acute monocytic leukemia	3	2
Chronic myelogenous leukemia	27	7
Myelofibrosis and myeloid metaplasia	7	5
Thrombocythemia	1	1
Acute lymphoblastic leukemia	8	4
Chronic lymphocytic leukemia	9	7
Lymphomas and correlated disorders	14	7
Total	143	

The variety in type and distribution of the described leukemias in the major case reports (with a more or less pronounced prevalence of acute myelogenous leukemia) may be due to several factors: a) difference in level of exposure; b) exposure in association with other agents, with toxic effects on hematopoietic tissues; c) individual responsiveness; and d) differences in the morphological interpretation of the hemopathological pictures, which vary with time, country, and training.

All of the previously mentioned reports deal with a limited series of cases, and therefore the cases are fragmented into a large number of reports (Table 1). In most of these reports, the causal relation between benzene exposure and leukemia is developed more from the history of the single cases, showing characteristic sequence, benzene exposure-myelotoxicity-leukemia, than from statistical and biological evaluation.

Moreover, the indirect results of one epidemiological investigation performed in Japan, were reported. Ishimaru and co-workers (*17*) examined 303 cases of leukemia that occurred among adult survivors of the atomic bomb explosions at Hiroshima and Nagasaki and compared them with 303 matched controls exposed to the same amount of ionizing radiation from the bomb. They found that the risk of leukemia was approximately 2.5 times higher among those with the history of a probable exposure to benzene or its derivatives and to medical X-rays.

Benzene was shown to be genotoxic to human blood cells. Increased rates of both stable and unstable chromosome changes have been described both in lymphocytes and bone marrow cells of patients with benzene hemopathy and in lymphocytes of workers with past exposure to benzene but without signs of poisoning (*18–24*). Chromosome damage from benzene can persist for years in long-lived lymphocytes and may result in the formation of abnormal cell clones in the absence of any sign of disease (*21,22*). Increased chromosome aberrations of blood lymphocytes have also been reported in workers exposed to less than 25 ppm of benzene (*25–27*). There have been a number of reports of cases of benzene pancytopenia in which the observed bone marrow cellular atypias were progressing toward acute leukemias.

The available experimental data prior to 1976, summarized in Table 2, are scanty and insubstantial (*28–31*). In IARC Monograph No. 7 (*32*), it was concluded that "benzene has been tested only in mice by subcutaneous injection and skin application. The data reported do not permit the conclusion that carcinogenic activity has been demonstrated."

Knowledge of Benzene Carcinogenicity from the Mid-1970s to the Present

A systematic integrated experimental project (the largest up to present) of long-term carcinogenicity bioassays on benzene began in April 1976 at the Bentivoglio (BT) Experimental Unit of the Bologna Institute of Oncology. The project was aimed at studying the carcinogenic effects of benzene administrated by different routes (ingestion and inhalation) at different daily doses/concentrations on animals of different species and strains (Sprague-Dawley and Wistar rats and Swiss and RF/J mice) and of different ages at the start of the treatment. As early as November-December 1977, preliminary results were published (*33*) showing that benzene was producing in Sprague-Dawley rats Zymbal gland carcinomas, an increase of other solid tumors and a marginal increase of malignant hemolymphoreticular neoplasias. Since then, the data of this project, have shown that benzene has carcinogenic effects when given by inhalation and by ingestion, it causes tumors in all tested animal species and strains, it is a multipotential carcinogen, as it produces a large variety of neoplasias, and there is a clear-cut dose-response relationship in benzene carcinogenesis. The data have been summarized, from 1977 to 1987, in various publications (*34–45*).

Further experimental bioassays were then performed in other laboratories. Bone marrow hyperplasia, thymic lymphoma (6/40), plasmacytoma (1/40), and leukemia (1/40) were reported in C57BL/6J mice exposed to air contain-

Table 2. Long-term carcinogenicity bioassays on benzene: available data until 1976.

Animals							
Species	Strain	Sex	No.	Treatment and other experimental details	Results	Observations	Reference
Mice	Albino	M,F	33 T[a]	SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, once weekly, for 17-21 weeks (total dose, about 1 mg/kg body weight)	8 Leukemias (from 4-8 months from the starting of the treatment)	No control group	(*28*)
Mice	F	?	30 T	SC injection of 0.001 mL of benzene in 0.1 mL of sesame oil	6 Leukemias (30%) (from 200-300 days of age)	Leukemia increase in treated animals is not statistically significant	(*29*)
			212 C		29 Leukemias (14%) (before 300 days of age)		
Mice	DBA2 C3H C57BL6	M	30 T 30 T 30 T	SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, for all life span	0 0 0	Maximum survival of DBA2, C3H and C57BL/6 mice: 730 days	(*30*)
	AKR	M	30 T		16 Leukemias (between the 7 and 16 months of treatment), 8 animals dead before the month 9 of treatment	Major survival of animals of control group	
			35 C		30 Leukemias		
Mice	Swiss	M,F	10 T	SC injection of 0.001 mL of benzene in 0.1 mL of olive oil, for 10 weeks	5 SC sarcomas (at autopsy, performed between days 162 and 253 from start of treatment)	2 Animals dead within the first 8 weeks of treatment No control group	(*31*)
Mice Rats Rabbits	Various	M,F	Very many	Skin applications	No effects	Nonsystematic and non-ad hoc planned experiments	Many

[a]T, treated; C, control.

ing 300 ppm benzene for 6 hr/day, 5 days/week, for 488 days as compared with an incidence of 2/40 lymphomas (nonthymic) in the controls (*46*). Myelogenous leukemia occurred in 2/40 CD-1 mice exposed to air containing 300 ppm benzene for 6 hr/day, 5 days/week, for life (*47*). The results of these two studies, because of the small number of animals used and the marginal increase of the observed hemolymphoreticular neoplasias, do not allow establishment of a positive association between the onset of the neoplasias and the benzene exposure.

Cronkite (*48,49*) showed that female mice exposed at 300 ppm (6 hr/day, 5 days/week for 16 weeks, after which exposures was stopped) exhibited increased incidence of leukemias (8/88 versus 20/89) largely due to thymic lymphomas (1/88 versus 10/89). In addition, Zymbal gland neoplasias (1/88 versus 16/89) and ovarian tumors (0/88 versus 8/89) were increased.

NTP performed a long-term carcinogenicity bioassay on F344/N rats and B6C3F$_1$ mice (*50*). Fifty male and fifty female/dose level were gavaged 5 days/week for 103 weeks. Doses of 0, 50 , 100, or 200 mg/kg body weight benzene in corn oil (5 mL/kg) were administered to male rats. Doses of 0, 25, 50, or 100 mg/kg benzene in corn oil were administered to female rats and to male and female mice. The results of this experiment confirm the findings of Bologna-BT project, i.e., that benzene is a multipotential carcinogen. A definite or marginally increased incidence of the following tumors was found in benzene-exposed animals of one or both sexes: tumors of Zymbal glands, oral cavity, and skin in rats; tumors of Zymbal glands, hemolymphoreticular tissues, lungs, Harderian glands, mammary glands, preputial glands, forestomach, ovary, and liver in mice.

Recent epidemiological studies of small cohorts exposed to benzene have demonstrated a causal association with leukemia (*51–58*). Infante et al. (*51*) reported an increased risk of leukemias among workers at three rubber hydrochloride plants (in two Ohio locations) who were exposed to benzene in the years 1940 to 1949. In that investigation, the vital status of 75% of the population was ascertained. The level of exposure was estimated to be not greater than the standards at that time would have allowed. Rinsky et al. (*53*) reported the findings of a more extensive investigation on the same rubber workers. The major findings of this important study may be summarized as follows: a) the workers were exposed to only one agent that has been associated with blood dyscrasias, i.e., benzene; b) exposure data, uncommonly complete throughout the study period (1940–1975), indicated that the exposures of the workers were, for the most part, within limits permissible at the time (these limits are not greatly higher than the current legal standards); and c) the vital status (alive, dead, and cause of death) for 98% of the study population was ascertained. There were seven deaths from leukemia (myelocytic or monocytic) among 748 workers; this rate is 5.6-fold greater than would be expected in a comparable population. For workers exposed 5 years or more, there was a 21-fold increased risk of death from leukemia. In 1986, Rinsky et al. (*57,58*) reexamined the updated mortality on the same cohorts and calculated a cumulative benzene exposure index (ppm × years) for each cohort member. These authors found that the standard mortality ratio (SMR) for leukemia was 328 and for multiple myeloma was 398. With stratification of the cohort by cumulative exposure, the SMRs for leukemia increased from 105 in workers with less than 40-ppm years exposure, to 314 in workers with 40- to 199-ppm years, to 1757 in those with from 200- to 399-ppm years, and to 4535 in those with 400-ppm years or more.

In 1985, Maltoni et al. reported the first experimental evidence of the carcinogenicity of benzene-correlated compounds, namely toluene and xylene (*44*). These results has been recently confirmed by the same authors by further experiments whose results are now in publication.

The history of benzene carcinogenicity is summarized in Table 3.

This report presents the up-to-date results of the experiments on benzene carcinogenicity performed by the Institute of Oncology of Bologna (BT experimental project).

Materials and Methods

The plan of the experiments is shown in Tables 4–10. The bioassay on RF/J Mice (Table 10) is part of a larger experiment aimed at studying the effect of 10% ethyl alcohol, administered instead of drinking water, on benzene carcinogenesis (Table 11). (The results of the whole experiment are now being submitted for publication.) Data on test compounds and test animals are presented in Table 12. Details on the conduct of the experiments are as follows:

- The animals were exposed by inhalation in air, 4 to 7 hr/day, 5 days/week, for 15 and 104 weeks. The chambers for inhalation exposure were stainless steel, with two glass doors, and measured 135 × 98 × 65 cm. The volume was 860 L. Continuous air flow provided 12 to 15 air changes per hour. Before introduction, air was filtered and the chamber arrangement was such that air flowed from one part of the chamber to the other with-

Table 3. History of benzene carcinogenicity.

Year	Report
1928	First report on an acute leukemia following benzene intoxication (*5*)
1960s	Reports of cases of leukemias among workers (mainly shoemakers) heavily exposed to benzene, in Italy (*8*)
1970s	Reports of cases of leukemias among workers (mainly shoemakers) heavily exposed to benzene, in Turkey (*15,16*)
1977	First evidence of carcinogenicity of benzene in experimental animals (rats) (*33*)
1977–1983	Experimental evidence showing that benzene is a multipotential carcinogen in rodents (rats and mice) (*43,44*)
1977–1986	Epidemiological evidence of benzene leukemogenicity among exposed workers in the U.S., also at low doses (*51,53,57,58*)
1983–1987	First experimental evidence of carcinogenicity of benzene correlated compounds (toluene and xylenes) (*44*)

Table 4. Plan of experiments on benzene carcinogenicity: experiment BT 901.[a]

		Sprague-Dawley rats, 13 weeks old at start		
Group no.	Dose	M	F	Total
I	250 mg/kg body weight	35	35	70
II	50 mg/kg body weight	30	30	60
III	0 (Controls)[b]	30	30	60
Total		95	95	190

[a]Exposure by ingestion (stomach tube) in olive oil, once daily, 4–5 days weekly, for 52 weeks. Duration of the biophase, life-span.
[b]Olive oil alone.

Table 5. Plan of experiments on benzene carcinogenicity: experiment BT 902.[a]

		Sprague-Dawley rats, 7 weeks old at start		
Group no.	Dose	M	F	Total
I	500 mg/kg body weight	40	40	80
II	0 (Controls)[b]	50	50	100
Total		90	90	180

[a]Exposure by ingestion (stomach tube) in olive oil, once daily, 4–5 days weekly, for 104 weeks. Duration of the biophase, life-span.
[b]Olive oil alone.

Table 6. Plan of experiments on benzene carcinogenicity: experiments BT 901, BT 902.[a]

	Treatment		Sprague-Dawley rats, 7 and 13 weeks old at start		
Experiment and group no.	Dose	Length, weeks	M	F	Total
BT 902 I	500 mg/kg body weight	104	40	40	80
BT 901 I	250 mg/kg body weight	52	35	35	70
BT 901 II	50 mg/kg body weight	52	30	30	60
BT 901 III	0 (Controls)[b]		30	30	60
BT 902 II	0 (Controls)[b]		50	50	100
Total			185	185	370

[a]Exposure by ingestion (stomach tube) in olive oil, once daily, 4–5 days weekly. Duration of the biophase, life-span.
[b]Olive oil alone.

out recirculation. The internal pressure was about 1 mm Hg less than that of the room where the chamber was situated, to avoid any possible contamination of the outside environment. Chambers were maintained at 21°C ± 3°C and at 50% ± 10% relative humidity. Lighting was provided by room light. The chambers were cleaned at monthly intervals. Exposure chambers were provided with a fixed point matrix for checking the distribution of the test substance. During treatment the distribution was continuously monitored by gas chromatographs.

- The animals were exposed by ingestion (stomach tube, made of stainless steel), once daily, 4 to 5 days/week, for 52 (Sprague-Dawley rats, RF/J mice), 78 (Swiss mice) and 104 weeks (Sprague-Dawley and Wistar rats).
- All the animals were kept under observation until spontaneous death.
- The status and behavior of the animals were examined 3 times daily.
- The animals were submitted to clinical examination for gross changes every 2 weeks.
- The animals were weighed every 2 weeks during treatment, and then every 8 weeks.
- Full necropsy was performed on all the animals; see below.
- The housing and the diet of the animals were the same adopted in the BT Experimental Unit during the last 15 years.
- All the experiments were performed with the same highly standardized procedures in order to allow comparison.

The tissues and organs submitted to histopathological examination were the following: subcutaneous lymph nodes, brain and cerebellum, pituitary, Zymbal glands, interscapular brown fat, salivary glands, Harderian glands, oral and nasal cavities (seven sections of the head), tongue, pharynx, thymus and mediastinal lymph nodes, lungs, diaphragm, liver, kidneys, adrenals, spleen, esophagus, mesenteric lymph nodes, stomach, various segments of the intestine, bladder, uterus, gonads, any other organs with pathological lesions, and, only for experiment BT 909, lachrymal and preputial glands.

Table 7. Plan of experiments on benzene carcinogenicity: experiments BT 4004,4006.[a]

Group no.	Treatment: Concentration	Treatment: Schedule	Sprague-Dawley rats, 13 weeks old, breeder (B) and 12-day embryo (E): Age	M	F	Total
I	200 ppm	4 hr/day, 5 days/week, 7 weeks	B		54[d] (22)[e]	54
		7 hr/day, 5 days/week, 12 weeks				
	300 ppm	7 hr/day, 5 days/week, 85 weeks[b]				
II	200 ppm	4 hr/day, 5 days/week, 7 weeks	E	75	65	140
		7 hr/day, 5 days/week, 12 weeks				
	300 ppm	7 hr/day, 5 days/week, 85 weeks[b]				
III	200 ppm	4 hr/day, 5 days/week, 7 weeks	E	70	59	129
		7 hr/day, 5 days/week, 8 weeks[c]				
IV	0 (Controls)		B		60[d] (24)[e]	60
V	0 (Controls)		E	158	149	307
Total				303	387	690

[a]Exposure by inhalation for 15 and 104 weeks. The embryos were exposed transplacentally during pregnancy, and the offspring were exposed concurrently during weaning by inhalation and possibly by ingestion via milk.
[b]Total period of exposure, 104 weeks.
[c]Total period of exposure, 15 weeks.
[d]Total breeders.
[e]Pregnant breeders.

Table 8. Plan of experiments on benzene carcinogenicity: experiment BT 907.[a]

Group no.	Dose	Wistar rats, 7 weeks old at start: M	F	Total
I	500 mg/kg body weight	40	40	80
II	0 (Controls)[b]	40	40	80
Total		80	80	160

[a]Exposure by ingestion (stomach tube) in olive oil, once daily, 4-5 days weekly, for 104 weeks. Duration of the biophase, life-span.
[b]Olive oil alone.

Table 9. Plan of experiments on benzene carcinogenicity: experiment BT 908.[a]

Group no.	Dose	Swiss mice, 7 weeks old at start: M	F	Total
I	500 mg/kg body weight	40	40	80
II	0 (Controls)[b]	40	40	80
Total		80	80	160

[a]Exposure by ingestion (stomach tube), in olive oil, once daily, 4-5 days weekly, for 78 weeks. Duration of the biophase, life-span.
[b]Olive oil alone.

Table 10. Plan of experiments on benzene carcinogenicity: experiment BT 909.[a]

Group no.	Dose	RF/J mice, 6 weeks old at start: M	F	Total
I	500 mg/kg body weight	45	40	85
II	0 (Controls)[b]	45	40	85
Total		90	80	170

[a]Exposure by ingestion (stomach tube), in olive oil, once daily, 4-5 days weekly, for 52 weeks. Duration of the biophase, life-span.
[b]Olive oil alone.

Table 11. Plan of experiments on benzene carcinogenicity: experiment BT 909.[a]

Group no.	Treatment	RF/J mice, 6 weeks old at start: M	F	Total
I	Benzene 500 mg/kg body weight in oil Ethyl alcohol[b]	45	40	85
II	Benzene 500 mg/kg body weight in oil Drinking water	45	40	85
III	Olive oil Ethyl alcohol	45	40	85
IV	Ethyl alcohol	45	40	85
V	Olive oil Drinking water	45	40	85
VI	Drinking water	45	40	85
Total		270	240	510

[a]Study on the effect of ethyl alcohol administration in drinking water on carcinogenic effect of benzene given by ingestion (stomach tube), in olive oil, for 52 weeks. Duration of the biophase, life-span.
[b]Ethyl alcohol instead of drinking water.

Table 12. Test compound and test animals.

Test compound	Purity	Vehicle	Test animals
Benzene	99.93%	(Ingestion experiments) extra-virgin olive oil, with no detectable levels of pesticides, supplied by Olearia Toscana.	Male and female Sprague-Dawley rats 7, 13 weeks old, and 12-day embryos at start of the experiments
Paraffin	0.06%		Male and female Wistar rats, 7 weeks old at start of the experiment
Toluene	0.01%		Male and female Swiss mice, 7 weeks old at start of the experiment
			Male and female RF/J mice, 6 weeks old at start of the experiment
			Rats (Sprague-Dawley, Wistar) and mice (Swiss) were of the breed currently used in the BT Experimental Unit for many years. The RF/J mice were supplied by Jackson Laboratory

Results

Experiment on Sprague-Dawley Rats (Tables 13–25)

The most frequent tumors in this strain of rats, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary tumors, malignant hemolymphoreticular neoplasias (leukemias), pheochromocytomas, and pheochromoblastomas. Moreover a variety of other miscellaneous tumors were also observed (*59*).

The administration of benzene by ingestion is associated with an increase of total malignant tumors and carcinomas of the Zymbal glands (with sebaceous and squamous patterns), oral cavity, nasal cavities, skin (of different histotypes), forestomach (together with an increase of acanthomas and dysplasias), and with liver angiosarcomas, and a marginal increase of carcinomas of the mammary glands, hepatomas, and leukemias.

The administration of benzene by inhalation is associated with an increase of total malignant tumors and carcinomas of the Zymbal glands and oral cavity, and with a marginal increase of carcinomas of the nasal cavities, mammary glands, and hepatomas.

Table 13. Experiment BT 901: incidence of total tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		% of animals bearing tumors		
Group no.	Dose, mg/kg	Sex	No. at start	TBMT[a]	MT[b]	No. of malignant tumors per 100 animals
I	250	M	35	37.1	20.0	22.8
		F	35	65.7	42.8	60.0
		M+F	70	51.4	31.4	41.4
II	50	M	30	33.3	3.3	3.3
		F	30	76.7	30.0	33.3
		M+F	60	55.0	16.7	18.3
III	Olive oil (Controls)	M	30	23.3	3.3	3.3
		F	30	60.0	23.3	23.3
		M+F	60	41.7	13.3	13.3

[a]Total benign and malignant tumors.
[b]Malignant tumors.

Table 14. Experiment BT 901: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

				% of animals bearing tumors				
		Animals		Mammary tumors				
Group no.	Dose, mg/kg	Sex	No. at start	BMT[a]	MT[b]	Leukemias	Pheochromocytomas	Pheochromoblastomas
I	250	M	35	5.7	—	11.4	2.9	
		F	35	45.7	20.0	2.9	2.9	—
		M+F	70	25.7	10.0	7.1	2.9	—
II	50	M	30	20.0	—	—	—	—
		F	30	73.3	13.3	6.7	3.3	—
		M+F	60	46.7	6.7	3.3	1.7	—
III	Olive oil (Controls)	M	30	3.3	—	—	3.3	—
		F	30	53.3	13.3	3.3	3.3	—
		M+F	60	28.3	6.7	1.7	3.3	—

[a]Benign and malignant tumors.
[b]Malignant tumors (carcinomas, carcinosarcomas).

Table 15. Experiment BT 901: incidence of Zymbal gland carcinomas, auricolar duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		*% of animals bearing tumors*				
Group no.	Dose, mg/kg	Sex	No. at start	Zymbal gland carcinomas	Auricolar duct carcinomas	Nasal cavity carcinomas	Oral cavity carcinomas	Total
I	250	M	35	—	—	—	—	—
		F	35	22.9	—	—	5.7	28.6
		M+F	70	11.4	—	—	2.9	14.3
II	50	M	30	—	—	—	—	—
		F	30	6.7	—	—	—	6.7
		M+F	60	3.3	—	—	—	3.3
III	Olive oil (Controls)	M	30	—	—	—	—	—
		F	30	—	—	—	—	—
		M+F	60	—	—	—	—	—

Table 16. Experiment BT 902: incidence of total tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

		Animals		% of animals bearing tumors		
Group no.	*Dose, mg/kg*	Sex	No. at start	TBMT[a]	MT[b]	No. of malignant tumors per 100 animals
I	500	M	40	92.5	90.0	170.0
		F	40	92.5	87.5	147.0
		M+F	80	92.5	88.7	158.7
II	Olive oil (Controls)	M	50	58.0	24.0	24.0
		F	50	60.0	20.0	22.0
		M+F	100	59.0	22.0	23.0

[a]Total benign and malignant tumors.
[b]*Malignant tumors.*

Table 17. Experiment BT 902: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

		Animals		% of animals bearing tumors: Mammary tumors				
Group no.	Dose, mg/kg	Sex	No. at start	BMT[a]	MT[b]	Leukemias	Pheochromo-cytomas	Pheochromo-blastomas
I	500	M	40	7.5	—	2.5	10.0	2.5
		F	40	32.5	17.5	7.5	5.0	—
		M+F	80	20.0	8.8	5.0	7.5	1.3
II	Olive oil (Controls)	M	50	4.0	—	6.0	40.0	—
		F	50	42.0	14.0	2.0	22.0	—
		M+F	100	23.0	7.0	4.0	31.0	—

[a]Benign and malignant tumors.
[b]Malignant tumors (carcinomas, sarcomas).

Table 18. Experiment BT 902: incidence of Zymbal gland carcinomas, auricolar duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

		Animals		% of animals bearing tumors				
Group no.	Dose, mg/kg	Sex	No. at start	Zymbal gland carcinomas	Auricolar duct carcinomas	Nasal cavity carcinomas	Oral cavity carcinomas	Total
I	500	M	40	45.0	—	7.5	52.5	105.0
		F	40	40.0	—	2.5	50.0	92.5
		M+F	80	42.5	—	5.0	51.2	98.8
II	Olive oil (Controls)	M	50	2.0	—	—	—	2.0
		F	50	—	—	—	—	—
		M+F	100	1.0	—	—	—	1.0

Table 19. Experiment BT 902: incidence of skin carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

Group no.	Dose, mg/kg	Animals: Sex	Animals: No. at start	% of animals bearing skin carcinomas
I	500	M	40	22.5
		F	40	–
		M+F	80	11.3
II	Olive oil (Controls)	M	50	–
		F	50	2.0
		M+F	100	1.0

Table 20. Experiment BT 902: incidence of hepatic tumors in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

Group no.	Dose, mg/kg	Animals: Sex	Animals: No. at start	% of animals bearing hepatic tumors: Hepatomas	% of animals bearing hepatic tumors: Angiosarcomas
I	500	M	40	7.5	5.0
		F	40	2.5	7.5
		M+F	80	5.0	6.2
II	Olive oil (Controls)	M	50	6.0	–
		F	50	–	–
		M+F	100	3.0	–

Table 21. Experiment BT 902: incidence of forestomach lesions in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

Group no.	Dose, mg/kg	Animals: Sex	Animals: No. at start	% of animals bearing forestomach lesions: Acanthomas and dysplasias	*In situ* carcinomas	Invasive carcinomas
I	500	M	40	25.0	–	2.5
		F	40	17.5	15.0	–
		M+F	80	21.3	7.5	1.3
II	Olive oil (Controls)	M	50	–	–	–
		F	50	–	–	–
		M+F	100	–	–	–

Table 22. Experiments BT 4004,4006: incidence of total tumors in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

Group no.	Treatment: Concentration, ppm	Treatment: Schedule	Animals: Age[a]	Animals: Sex	Animals: No. at start	% of animals bearing tumors: TBMT[b]	% of animals bearing tumors: MT[c]	No. of malignant tumors per 100 animals
I	200	4 hr/day, 5 days/week, 7 weeks	B	F	54	70.4	27.8	29.6
		7 hr/day, 5 days/week, 12 weeks						
	300	7 hr/day, 5 days/week, 85 weeks						
II	200	4 hr/day, 5 days/week, 7 weeks	E	M	75	56.0	30.7	37.3
				F	65	78.5	58.5	78.5
		7 hr/day, 5 days/week, 12 weeks		M+F	140	66.4	43.6	56.4
	300	7 hr/day, 5 days/week, 85 weeks						
III	200	4 hr/day, 5 days/week, 7 weeks	E	M	70	52.8	28.6	31.4
				F	59	78.0	45.8	50.8
		7 hr/day, 5 days/week, 8 weeks		M+F	129	64.3	36.4	40.3
IV	0 (Controls)		B	F	60	58.3	15.0	16.7
V	0 (Controls)		E	M	158	44.9	17.1	18.3
				F	149	78.5	17.4	17.4
				M+F	307	61.2	17.3	17.9

[a]B, breeders, E, embryos.
[b]Total benign and malignant tumors.
[c]Malignant tumors.

Table 23. Experiments BT 4004,4006: incidence of mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

	Treatment		Animals			% of animals bearing tumors				
						Mammary tumors				
Group no.	Concentration, ppm	Schedule	Age[a]	Sex	No. at start	BMT[b]	MT[c]	Leukemias	Pheochromo-cytomas	Pheochromo-blastomas
I	200	4 hr/day, 5 days/week, 7 weeks	B	F	54	55.5	11.1	—	7.4	—
		7 hr/day, 5 days/week, 12 weeks								
	300	7 hr/day, 5 days/week, 85 weeks								
	200	4 hr/day, 5 days/week,	E	M	75	8.0	—	8.0	8.0	1.3
		7 weeks		F	65	53.8	13.8	—	6.1	1.5
		7 hr/day, 5 days/week, 12 weeks		M+F	140	29.3	6.4	4.3	7.1	1.4
	300	7 hr/day, 5 days/week, 85 weeks								
III	200	4 hr/day, 5 days/week,	E	M	70	11.4	—	5.7	20.0	2.9
		7 weeks		F	59	62.1	13.6	6.8	8.5	—
		7 hr/day, 5 days/week, 8 weeks		M+F	129	34.1	6.2	6.2	14.7	1.6
IV	0 (Controls)		B	F	60	40.0	3.3	3.3	18.3	—
V	0 (Controls)		E	M	158	7.0	1.9	7.6	20.2	0.6
				F	149	56.4	5.4	0.7	18.8	0.7
				M+F	307	30.9	3.6	4.2	20.8	0.6

[a]B, breeders, E, embryos.
[b]Benign and malignant tumors.
[c]Malignant tumors (carcinomas, sarcomas).

Table 24. Experiments BT 4004,4006: incidence of Zymbal gland carcinomas, auricolar duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

	Treatment		Animals			% of animals bearing tumors				
Group no.	Concentration, ppm	Schedule	Age[a]	Sex	No. at start	Zymbal gland carcinomas	Auricolar duct carcinomas	Nasal cavity carcinomas	Oral cavity carcinomas	Total
I	200	4 hr/day, 5 days/week, 7 weeks	B	F	54	5.5	—	1.8	3.7	11.1
		7 hr/day, 5 days/week, 12 weeks								
	300	7 hr/day, 5 days/week, 85 weeks								
II	200	4 hr/day, 5 days/week,	E	M	75	8.0	—	1.3	1.3	10.7
		7 weeks		F	65	12.3	—	3.1	15.4	30.8
		7 hr/day, 5 days/week, 12 weeks		M+F	140	10.0	—	2.1	7.9	20.0
	300	7 hr/day, 5 days/week, 85 weeks								
III	200	4 hr/day, 5 days/week,	E	M	70	5.7	—	1.4	2.8	10.0
		7 weeks		F	59	1.7	—	1.7	10.2	13.6
		7 hr/day, 5 days/week, 8 weeks		M+F	129	3.9	—	1.6	6.2	11.6
IV	0 (Controls)		B	F	60	1.7	—	—	—	1.7
V	0 (Controls)		E	M	158	1.3	—	—	—	1.3
				F	149	—	—	—	—	—
				M+F	307	0.7	—	—	—	0.7

[a]B, breeders, E, embryos.

Table 25. Experiments BT 4004,4006: incidence of hepatic tumors in Sprague-Dawley rats exposed to benzene by inhalation for 15 and 104 weeks.

	Treatment		Animals			% of animals bearing tumors	
Group no.	Concentration, ppm	Schedule	Age[a]	Sex	No. at start	Hepatomas	Angiosarcomas
I	200	4 hr/day, 5 days/week, 7 weeks	B	F	54	1.8	–
		7 hr/day, 5 days/week, 12 weeks					
	300	7 hr/day, 5 days/week, 85 weeks					
II	200	4 hr/day, 5 days/week,	E	M	75	2.7	–
		7 weeks		F	45	10.8	–
		7 hr/day, 5 days/week,		M+F	140	6.4	–
		12 weeks					
	300	7 hr/day, 5 days/week, 85 weeks					
III	200	4 hr/day, 5 days/week,	E	M	70	2.8	–
		7 weeks		F	59	8.5	–
		7 hr/day, 5 days/week,		M+F	129	5.4	–
		8 weeks					
IV	0 (Controls)		B	F	60	–	–
V	0		E	M	158	0.6	–
	(Controls)			F	149	–	–
				M+F	307	0.3	–

[a]B, breeders, E, embryos.

Experiment on Wistar Rats (Tables 26–28)

The most frequently expected tumors in this strain of rats, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary tumors, leukemias, pheochromocytomas, and pheochromoblastomas. Moreover, a variety of other miscellaneous tumors were also observed (*59*).

The administration of benzene by ingestion is associated with an increase of total malignant tumors and carcinomas of Zymbal glands, oral cavity, and nasal cavities.

Table 26. Experiment BT 907: incidence of total tumors in Wistar rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

		Animals		No. of animals bearing tumors		
Group no.	Dose, mg/kg	Sex	No. at start	TBMT[a]	MT[b]	No. of malignant tumors per 100 animals
I	500	M	40	57.5	47.5	60.0
		F	40	67.5	52.5	70.0
		M+F	80	62.5	50.0	65.0
II	Olive oil	M	40	75.0	20.0	25.0
	(Controls)	F	40	85.0	25.0	30.0
		M+F	80	80.0	22.5	27.5

[a]Total benign and malignant tumors.
[b]Malignant tumors.

Table 27. Experiment BT 907: incidence of mammary tumors, leukemias, pheochromocytomas and pheochromoblastomas in Wistar rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

				% of animals bearing tumors				
		Animals		Mammary tumors				
Group no.	Dose, mg/kg	Sex	No. at start	BMT[a]	MT[b]	Leukemias	Pheochromocytomas	Pheochromoblastomas
I	500	M	40	2.5	–	5.0	2.5	–
		F	40	42.5	5.0	10.0	–	–
		M+F	80	22.5	2.5	7.5	1.2	–
II	Olive oil	M	40	10.0	–	2.5	5.0	–
	(Controls)	F	40	55.0	7.5	7.5	5.0	–
		M+F	80	32.5	3.7	5.0	5.0	–

[a]Benign and malignant tumors.
[b]Malignant tumors (carcinomas).

Table 28. Experiment BT 907: incidence of Zymbal gland carcinomas, auricolar duct carcinomas, nasal cavities carcinomas, and oral cavity carcinomas in Wistar rats exposed to benzene by ingestion (stomach tube) for 104 weeks.

		Animals		% of animals bearing tumors				
Group no.	*Dose, mg/kg*	*Sex*	*No. at start*	*Zymbal gland carcinomas*	*Auricolar duct carcinomas*	*Nasal cavity carcinomas*	*Oral cavity carcinomas*	*Total*
I	500	M	40	17.5	–	5.0	5.0	27.5
		F	40	15.0	–	2.5	10.0	27.5
		M+F	80	16.2	–	3.7	7.5	27.5
II	Olive oil	M	40	–	–	–	2.5	2.5
	(Controls)	F	40	–	2.5	–	–	2.5
		M+F	80	–	1.2	–	1.2	2.5

Experiment on Swiss Mice (Tables 29–33)

The most frequently expected tumors in this strain of mice, on the basis of the literature and of the historical controls of the BT Experimental Unit, are mammary carcinomas (in females), lung tumors, leukemias, and hepatomas. Moreover, a variety of other miscellaneous tumors were also observed (*59*).

The administration of benzene by ingestion is associated with an increase of total malignant tumors, carcinomas of the mammary glands, lung tumors (adenomas, adenomas in deviation, and adenocarcinomas), and carcinomas of the Zymbal glands (together with an increase of dysplasias).

Table 29. Experiment BT 908: incidence of total tumors in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

		Animals		No. of animals bearing tumors		
Group no.	Dose, mg/kg	Sex	No. at start	TBMT[a]	MT[b]	No. of malignant tumors per 100 animals
I	500	M	40	60.0	35.0	40.0
		F	40	80.0	70.0	80.0
		M+F	80	70.0	52.5	60.0
II	Olive oil	M	40	37.5	22.5	22.5
	(Controls)	F	40	40.0	27.5	27.5
		M+F	80	38.7	25.0	25.0

[a]Total benign and malignant tumors.
[b]Malignant tumors.

Table 30. Experiment BT 908: incidence of mammary carcinomas, pulmonary tumors, leukemias, and hepatomas in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

		Animals		% of animals bearing tumors			
Group no.	Dose, mg/kg	Sex	No. at start	Mammary carcinomas	Pulmonary tumors	Leukemias	Hepatomas
I	500	M	40	–	42.5	12.5	7.5
		F	40	47.5	37.5	20.0	–
		M+F	80	23.7	40.0	16.2	3.8
II	Olive oil	M	40	2.5	7.5	12.5	5.0
	(Controls)	F	40	5.0	10.0	20.0	–
		M+F	80	3.7	8.7	16.2	2.5

Table 31. Experiment BT 908: incidence of pulmonary lesions of oncological interest in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

		Animals		% of animals bearing pulmonary lesions				
Group no.	Dose, mg/kg	Sex	No. at start	Total	Adenomatous hyperplasia-early adenomas	Adenomas	Adenomas[a]	Adenocarcinomas
I	500	M	40	42.5	2.5	22.5	15.0	2.5
		F	40	37.5	–	22.5	15.0	–
		M+F	80	40.0	1.2	22.5	15.0	1.2
II	Olive oil	M	40	7.5	–	5.0	2.5	–
	(Controls)	F	40	10.0	–	10.0	–	–
		M+F	80	8.7	–	7.5	1.2	–

[a]Adenomas in deviation.

Table 32. Experiment BT 908: incidence of Zymbal gland carcinomas and correlated precancerous lesions in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

		Animals		% of animals bearing lesions		
Group no.	Dose, mg/kg	Sex	No. at start	Total	Dysplasias	Carcinomas
I	500	M	40	17.5	7.5	10.0
		F	40	12.5	10.0	2.5
		M+F	80	15.0	8.7	6.2
II	Olive oil (Controls)	M	40	–	–	–
		F	40	–	–	–
		M+F	80	–	–	–

Table 33. Experiment BT 908: incidence of hepatic tumors in Swiss mice exposed to benzene by ingestion (stomach tube) for 78 weeks.

		Animals		% of animals bearing hepatic tumors	
Group no.	Dose, mg/kg	Sex	No. at start	Hepatomas	Angiosarcomas
I	500	M	40	7.5	2.5
		F	40	–	–
		M+F	80	3.7	1.2
II	Olive oil (Controls)	M	40	5.0	–
		F	40	–	–
		M+F	80	2.5	–

Experiment on RF/J Mice (Tables 34–37)

The most frequently expected tumors of this strain of mice, on the basis of the literature and of our experimental experience, are mammary carcinomas, pulmonary tumors, and leukemias. The most frequent histotype of hemolymphoreticular malignant neoplasias are lymphoblastic lymphosarcomas (much greater percentage) and lymphoblastic lymphosarcomas with histocytic component.

The administration of benzene by ingestion is associated with an increase of total malignant tumors, mammary carcinomas, lung tumors (adenomas, adenomas in deviation, and adenocarcinomas), and leukemias. In the treated animals the number of pulmonary tumors per tumor-bearing animal is greatly enhanced (Table 37).

Table 34. Experiment BT 909: incidence of total tumors in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		No. of animals bearing tumors		
Group no.	Dose, mg/kg	Sex	No. at start	TBMT[a]	MT[b]	No. of malignant tumors per 100 animals
I	500	M	45	73.3	57.8	60.0
		F	40	85.0	75.0	85.0
		M+F	85	78.8	65.9	71.8
II	Olive oil (Controls)	M	45	40.0	42.2	44.4
		F	40	50.0	37.5	37.5
		M+F	85	44.7	40.0	41.2

[a]Total benign and malignant tumors.
[b]Malignant tumors.

Table 35. Experiment BT 909: incidence of mammary carcinomas, pulmonary tumors, leukemias, and hepatomas in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		% of animals bearing tumors			
Group no.	Dose, mg/kg	Sex	No. at start	Mammary carcinomas	Pulmonary tumors	Leukemias	Hepatomas
I	500	M	45	–	51.1	57.8	–
		F	40	22.5	45.0	60.0	–
		M+F	85	10.6	48.2	58.8	–
II	Olive oil (Controls)	M	45	–	11.1	37.8	–
		F	40	2.5	7.5	35.0	–
		M+F	85	1.2	9.4	36.5	–

Table 36. Experiment BT 909: incidence of pulmonary lesions of oncological interest in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		% of animals bearing lesions[a]					
					Tumors				
Group no.	Dose, mg/kg	Sex	No. at start	Simple adenomatous hyperplasia	Total	Adenomatous hyperplasia-early adenomas	Adenomas	Adenomas[b]	Adenocarcinomas
I	500	M	45	—	51.1	2.2	20.0	28.9	—
		F	40	5.0	45.0	—	30.0	7.5	2.5
		M+F	85	2.4	48.2	1.2	24.7	18.8	1.2
II	Olive oil (Controls)	M	45	2.2	11.1	—	8.9	2.2	—
		F	40	—	7.5	—	7.5	—	—
		M+F	85	1.2	9.4	—	8.2	1.2	—

[a]For each tumor only the gravest lesion was counted.
[b]Adenomas in deviation.

Table 37. Experiment BT 909: incidence of total pulmonary lesions of oncological interest in RF/J mice exposed to benzene by ingestion (stomach tube) for 52 weeks.

		Animals		No. of pulmonary lesions/100 animals[a]					
					Tumors				
Group no.	Dose, mg/kg	Sex	No. at start	Simple adenomatous hyperplasia	Total	Adenomatous hyperplasia-early adenomas	Adenomas	Adenomas[b]	Adenocarcinomas
I	500	M	45	24.4	226.7	28.9	142.2	55.6	—
		F	40	17.5	80.0	5.0	65.0	7.5	2.5
		M+F	85	21.2	157.6	17.6	105.9	32.9	1.2
II	Olive oil (Controls)	M	45	2.2	15.6	—	13.3	2.2	—
		F	40	—	10.0	—	10.0	—	—
		M+F	85	1.2	12.9	—	11.8	1.2	—

[a]All different lesions present in each animal were counted.
[b]Adenomas in deviation.

Multipotential Carcinogenicity, Dose-Response, and Effect of Age

A variety of tumors is associated to benzene exposure in the animals of all tested species and strains (Table 38).

A dose-response relationship was seen in the experiment with Sprague-Dawley rats. This relation appears particularly marked in the case of carcinomas of Zymbal glands, oral cavity, and nasal cavities when considered either singularly or together (Table 39).

An enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life (Table 24-28).

Table 38. Tumor associated to benzene exposure on the basis of the BT experimental project.

Tumors	Sprague-Dawley rat, Ingestion	Sprague-Dawley rat, Inhalation	Wistar rat, ingestion	Swiss mouse, ingestion	RF/J mouse, ingestion
Total malignant tumors	+	+	+	+	+
Carcinomas of Zymbal gland	+	+	+	+	
Carcinomas of oral cavity	+	+	+		
Carcinomas of nasal cavities	+	(+)[a]	+		
Carcinomas of the skin	+				
Carcinomas of the forestomach	+				
Carcinomas of the mammary gland	(+)	(+)		+	+
Hepatomas	(+)	(+)			
Angiosarcomas of the liver	+				
Hemolymphoreticular neoplasias	(+)				+
Tumors of the lung				+	+

[a]Weak evidence.

Table 39. Experiments BT 901, BT 902: incidence of Zymbal gland carcinomas, nasal cavities carcinomas, and oral cavities carcinomas in Sprague-Dawley rats exposed to benzene by ingestion (stomach tube) for 52 and 104 weeks.

Experiment and group no.	Treatment		Animals		% of animals bearing tumors			
	Dose, mg/kg	Length, weeks	Sex	No. at start	Zymbal gland carcinomas	Nasal cavity carcinomas	Oral cavity carcinomas	Total
BT 902	500	104	M	40	45.0	7.5	52.5	105.0
I			F	40	40.0	2.5	50.0	92.5
			M+F	80	42.5	5.0	51.2	98.8
BT 901	250	52	M	35	—	—	—	—
I			F	35	22.9	—	5.7	28.6
			M+F	70	11.4	—	2.9	14.3
BT 901	50	52	M	30	—	—	—	—
II			F	30	6.7	—	—	6.7
			M+F	60	3.3	—	—	3.3
BT 901	Olive oil	52	M	30	—	—	—	—
III	(Controls)		F	30	—	—	—	—
			M+F	60	—	—	—	—
BT 902	Olive oil	104	M	30	2.0	—	—	2.0
II	(Controls)		F	30	—	—	—	—
			M+F	60	1.0	—	—	1.0

Conclusions

The experiments performed at the Bologna Institute of Oncology on benzene carcinogenesis have shown that benzene is a strong carcinogen on experimental animals; it is carcinogenic on four different types of experimental animals, i.e., Sprague-Dawley and Wistar rats, and Swiss and RF/J mice. Exposure to benzene is associated with an enhanced incidence of a variety of tumors; therefore, benzene must be considered a multipotential carcinogen. The neoplastic response associated with benzene exposure varies in the different types of tested animals. Benzene has carcinogenic effects when given both by inhalation and by ingestion. The carcinogenic effects of benzene increase by increasing the doses (daily dose, length of treatment). There is a high response when treatment is started during embryonal life.

The experimental research may still improve our knowledge of benzene carcinogenicity and correlated problems. At present, in our opinion, the following experimental research deserves full priority: a) studies on the carcinogenic effects of minimal doses of benzene (in the range of the present allowable levels), delivered by inhalation, to large groups of animals (mega-experiments); b) carcinogenicity studies on chemical mixtures containing benzene (fuels); c) carcinogenicity studies on chemically correlated and/or alternative compounds, i.e., toluene, xylenes, trimethylbenzenes, ethylbenzene, etc. Studies on these three fields of research are now ongoing or planned at the Bologna Institute of Oncology. Moreover, comprehensive epidemiological investigations on population groups exposed to benzene extended to all types of malignancies, with particular regard to lung carcinomas (on the basis of our recent experimental results) must be undertaken without delay.

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